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## INVESTIGATIVE REPORT

# Ceramides and Barrier Function in Healthy Skin

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**Lipids in the stratum corneum are key components in the barrier function of the skin. Changes in lipid composition related to eczematous diseases are well known, but limited data are available on variations within healthy skin. The objective of the present study was to compare ceramide subgroups and ceramide/cholesterol ratios in young, old, male and female healthy skin. A total of 55 participants with healthy skin was included in the study. Lipid profiles were correlated with transepidermal water loss and with information on dry skin from a questionnaire including 16 people. No statistically significant differences were found between young and old skin for ceramide subgroups or ceramide/cholesterol ratios, and there was no statistically significant correlation between answers about dry skin and ceramide levels. Interestingly, a statistically significant higher ceramide/cholesterol ratio was found for men than for women ( $p=0.02$ ). Key words: age; ceramides; ceramide/cholesterol ratio; gender; stratum corneum; TEWL.**

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The barrier function of the skin is located in the stratum corneum (SC), where the majority of the very limited transport of substances occurs between the corneocytes in the lipid bilayers (1, 2). The lipid bilayers are composed of ceramides, cholesterol and free fatty acids, and the ceramide profile in particular has been related to the barrier function of the skin. Ceramide 1 and ceramide 3 have been reported to be reduced in the SC of patients with atopic eczema (3–6), and some, but not all, studies have reported a negative correlation between ceramide 3 and transepidermal water loss (TEWL) (3, 7). Limited data are available on variation in skin lipids and ceramide profile due to physiological parameters such as age and gender. Two studies have found overall reduced lipid content in aged human skin (4, 8), but research on healthy skin is very limited (2).

Identification of filaggrin mutations related to atopic eczema, ichthyosis and other eczematous diseases

(9–13) has renewed interest in research into skin barrier function, including skin lipids. However, more information about the amount of SC lipids in normal skin is required in order to obtain a better understanding of the diseased skin. The aim of the present study was to evaluate the ceramide profile of healthy volunteers in relation to age and gender, and to correlate ceramide profile with TEWL and clinical perception of dry skin.

## MATERIALS AND METHODS

A total of 55 healthy volunteers was included in the study (19 men and 36 women). Thirty-two were <40 years of age (median age 26 years, age range 18–39 years), 5 were between 40 and 60 years (median 51 years) and 18 were >60 years of age (median age 76 years, age range 61–88 years). Participants were enrolled after responding to posters at the local library and educational centre, and had no history of any major skin diseases. The study was approved by the local ethics committee (SJ-7, 13986).

### Methods

SC was collected from all participants, using cyanoacrylate methods (14). Participants were instructed not to use any moisturizers on the day of the examination. The mid-volar forearm was wiped with acetone to eliminate contamination with surface lipids. A drop of cyanoacrylate tissue-glue (LiquiBand®, Medlogix Global Ltd, Plymouth, UK) was placed on a glass slide and held tightly against the skin for 2 min, and then removed. The slide was stored at –80°C until further analysis by high-performance thin layer chromatography (HPTLC). For HPTLC the skin lipids were separated on silica-coated HPTLC plates, due to the difference in the strength of interaction between the different lipids and the silica gel using a solvent mixture of chloroform:methanol:acetic acid (190:9:1(v:v:v)). The samples were compared with standard curves made from ceramide 5 and cholesterol included on the plate. After separation, the plates were dried, stained with the fluorescent probe primuline and the components were quantified through determination of fluorescence intensity, as described in detail elsewhere (14).

For ceramides we use the simple nomenclature (ceramide 1–9); however, in Fig. 1 for clarity we have included the nomenclature suggested by Motta et al. (15).

TEWL measurements were obtained at the volar forearm from 31 of the volunteers. Measurements were performed on the opposite forearm to where the cyanoacrylate strip was taken, in accordance with guidelines (16).

Data on the clinical perception of dry skin was obtained from 16 healthy participants (median age 32 years, age range 18–51 years; 5 males, 11 females). Individual dryness of the skin, during the last week, expressed on a visual analogue scale (VAS)-score (0=no dry skin and 10=severely dry skin), and asked “Do you have dry skin (yes/no)? If yes, do you have dry

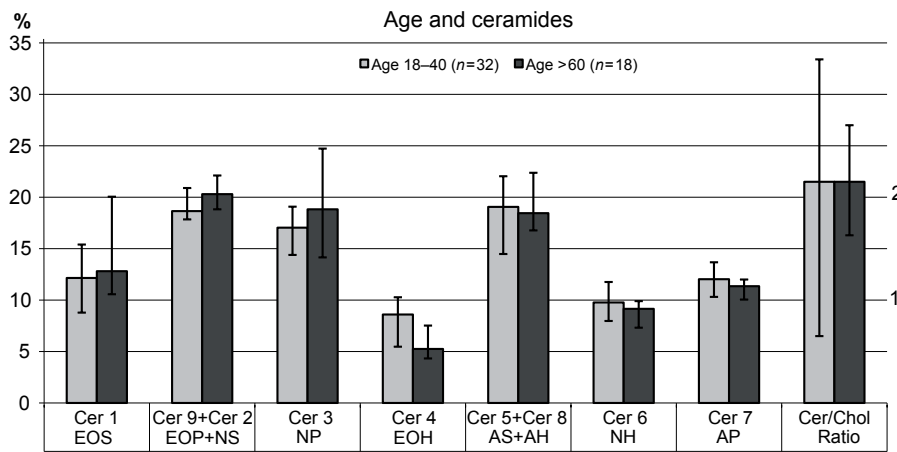


Fig. 1. Comparison of the percentage median stratum corneum ceramide values for healthy young skin and healthy old skin. Percentiles 1 and 3 are shown for each median value. No statistically significant differences were measured. The y-axis represents the median percentage distribution of ceramide 1–9 and is indicated *left*, while the ceramide/cholesterol (Cer/Chol) ratio is indicated *right*. Motta's nomenclature (15) is added for each ceramide: A:  $\alpha$ -hydroxy fatty acid; EO: ester-linked  $\omega$ -hydroxyl acid; N: non-hydroxy fatty acid; P: phytosphingosine; S: sphingosine; H: 6-hydrosphingosine.

skin only in the winter or both summer and winter?" (Questions were asked and samples were obtained in October).

#### Statistics

For comparison of differences between groups with respect to age and gender, the Mann-Whitney test was used. For correlation studies, the Spearman's rank correlation coefficient was used.  $p$ -values  $< 0.05$  were considered statistically significant.

## RESULTS

Results of the comparison of the ceramide profile in young and old skin are illustrated in Fig. 1. No statistically significant differences in ceramides or ceramide/cholesterol ratios ( $p = 0.57$ ) were found in SC from young ( $< 40$  years) and old ( $> 60$  years) participants.

Ceramide profiles from the SC of male and female volunteers are shown in Fig. 2. There was a significant difference between the ceramide/cholesterol ratios for men and women (median men 2.0; median women 2.3;  $p = 0.02$ ). No statistically significant differences were found between any of the ceramide subgroups between the age-matched men and women.

The relationship with TEWL for ceramide 1 and 3 is shown in Fig. 3. No significant correlation was found ( $p = 0.76$  and  $p = 0.57$ , respectively). No correlation with TEWL was found for any of the other ceramide classes either, and no correlation between TEWL and the ceramide/cholesterol ratio ( $p = 0.60$ ) was found.

The questionnaire on dry skin showed no significant differences in ceramide classes from volunteers with dry and normal skin, respectively, and no significant correlation between the VAS-score (0–10) and the different ceramides was found. One in 5 males reported dry skin, compared with 7 of the 11 females.

## DISCUSSION

Ceramides are thought to play a major role in maintaining the efficient barrier function of the SC. Ceramide 1, in particular, is thought to be of importance in the organization of lipids in the SC (1, 17, 18). However, knowledge of the different ceramides and ceramide/cholesterol ratio in healthy skin is very limited, but is nevertheless important for a better understanding of diseased skin.

No statistically significant difference was found in this study between young and old skin with respect to ceramide profile. Two other groups have previously studied ceramide changes with respect to age. One group examined Japanese volunteers and used roughly the same cyanoacrylate method as ours. They found a decline in the total ceramide content with increasing age in SC, but did not differentiate among the subgroups of ceramides (4), thus the results of this study are difficult to compare with our results, in which the ceramide levels are given as a ratio of cholesterol. The other group used tape-stripping on female Caucasians; they

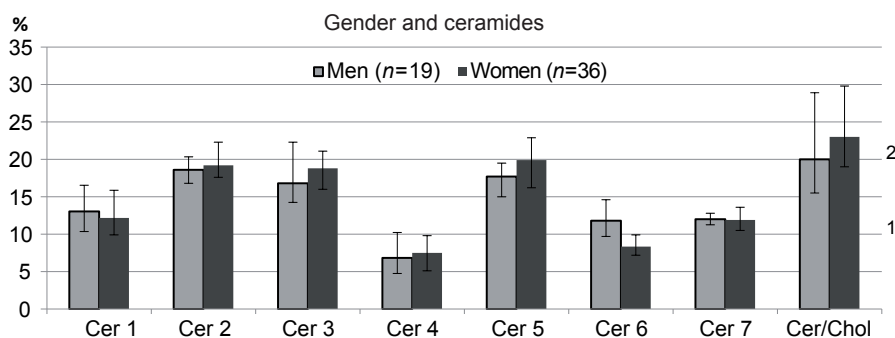


Fig. 2. Comparison of the percentage median stratum corneum ceramide values for healthy men and women and ceramide/cholesterol ratios (Cer/Chol). Percentiles 1 and 3 are shown for each median value. No statistically significant differences were found for the ceramide subgroups, but there was a statistically significant difference in the Cer/Chol ratio between men and women. The y-axis represents the median percentage distribution of ceramide 1–7 and is indicated *left*, while the Cer/Chol ratio is indicated *right*.

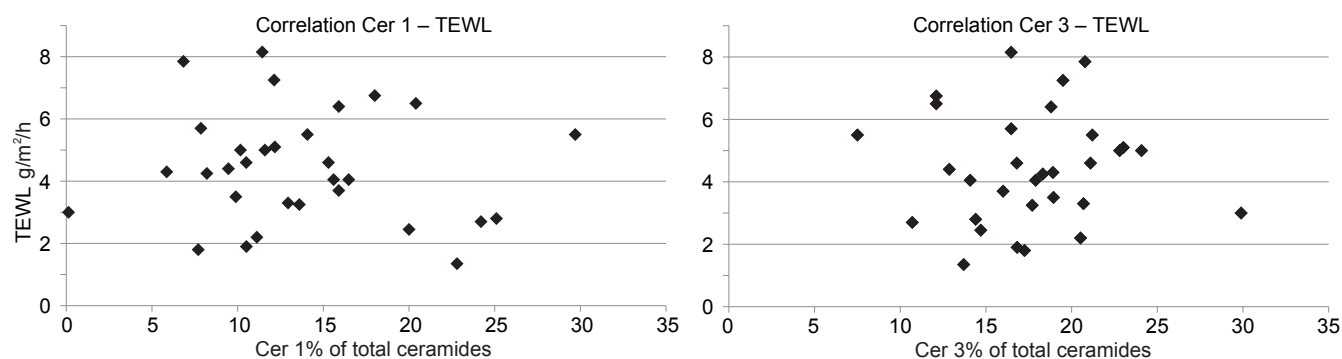


Fig. 3. Lack of correlation of transepidermal water loss (TEWL) and percentage of ceramide 1 and ceramide 3, respectively,  $n=31$ .  $p$ -values are 0.76 and 0.57, respectively.

examined the hands and face, and divided the females into age groups of 21–30, 31–40 and 41–50 years (8). They found a decreased level of all major lipid classes with increasing age, but, like us, they did not find any change in the ratio of either the total ceramide level or in any subgroups of ceramides. An overall reduction in lipids in aged SC has also been reported in a recent study, and was explained by the increasing pH of aged skin (19). However, this explanation is controversial, because conflicting results on pH and skin age exist (20). In conclusion, it appears that there is no correlation between clinically dry skin in elderly people and any ceramide subgroups or ceramide/cholesterol ratio.

It has been demonstrated that the skin response to sodium lauryl sulphate is influenced by the menstrual cycle (21), but whether this correlates with a cyclical change in lipid levels has not been examined; however, since the women in the present study would most probably have been at different stages of the menstrual cycle, the impact of this would be minimal. In addition, there is no difference between genders when evaluated by basal TEWL (16). One group found no statistically significant differences in ceramide subgroups between genders for adult skin (22). They did, however, find differences between the skin of pre-pubertal girls and adults (22). Another group found differences in the abdominal skin, with an increase in total ceramides for men compared with women (23). However, they examined only 3 male subjects, thus caution should be exercised in interpreting gender differences. In the present study no significant differences were found in ceramide profile in relation to gender, but there was a significant difference in the ceramide/cholesterol ratio, whereby men had the lowest ratio, which was closer to the atopic ratio (2). It could be argued that this difference may be due to the use of moisturizers; however, we tried to overcome this by instructing participants not to use moisturizer on the examined area on the day of examination, and a recent study found no difference in SC lipids after one week of two daily applications of a moisturizer compared with untreated control (Jungersted JM 2010, unpublished data).

TEWL has previously been reported to correlate negatively with the amount of ceramide 3 in atopic SC (3). Another group found no correlation between TEWL and total amount of ceramides (7). The present study did not support a correlation between any of the ceramide subgroups and TEWL in a group of healthy volunteers.

Including the above-mentioned observed gender differences with respect to experiences of dry skin, no statistically significant correlations were found between the answers to the questionnaire and the ceramides or TEWL.

This study focused on ceramides in the SC of healthy volunteers, as a basis for better understanding of diseased skin. In future research it would be interesting to determine what happens to SC lipids under different circumstances, such as occlusion, and during different treatment regimes.

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